Renewal Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

- XenTari® WG -

Volume 3 – B.8 Fate and behavior in the environment

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

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B.8 Fate and behaviour in the environment

Bacillus thuringiensis subsp. *aizawai* strains ABTS-1857, GC-91 were included in Annex I to Directive 91/414/EEC (2008/113/EC) on 1 May 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009 in accordance with Commission Implementing Regulation (EU) No 540/2011 as amended by Commission Implementing Regulation (EU) No 541/2011.

European Food Safety Authority (EFSA) revised the draft review report submitted by the European Commission and EFSA's conclusion was published in the peer review (EFSA Journal 2013; 11(1): 3063).

Italy was designated rapporteur Member State and the DAR was issued in 2008.

The plant protection product XenTari® WG is considered representative of uses of the MPCA *Bacillus thuringiensis* subsp. *aizawai* strain ABTS 1857 for the purposes of renewal of the approval of the MPCA under EC Regulation 1107/2009 according to Regulation EU 283/2013.

This document reviews the information and modelling relating to the environmental fate and behaviour for the plant protection product XenTari® WG a water dispersable granule (WG) formulation containing the MPCA *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 (54%L) for control of various insect pests.

Environmental exposure following the use of the representative product XenTari® WG has been assessed.

The initial predicted environmental densities (PED) of the formulated product XenTari® WG and the MPCA in soil, groundwater, surface water and sediment are provided where relevant, together with long term actual and time weighted average (TWA) concentrations, where appropriate.

Environmental fate studies on the formulation XenTari® WG have not been performed since it is possible to extrapolate from data obtained with the individual MPCA. Furthermore, the impact of formulants is limited to the short-term effects and their influence on long-term processes, such as degradation and distribution is negligible. Therefore for the purposes of this risk assessment it is assumed that formulants do not influence the fate and behaviour of the MPCA in the environment and are not considered further.

B.8.1 Persistence and multiplication

Use of the formulated product XenTari WG can potentially lead to amounts reaching soil, therefore the fate and behaviour in soil of XenTari WG is addressed. The formulated product XenTari WG contains the MPCA *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857.

B.8.1.1 Soil

The fate and behaviour of the MPCA in soil is evaluated under Point B.8.1.1 of the corresponding active substance dossier. As it is possible to extrapolate the behaviour of the MPCA resulting from use of the formulated product XenTari WG from the information on the MPCA itself, additional information investigating the rate of degradation in soil of the formulated product is not required.

Predicted Environmental Densities in Soil (PEDsoil)

XenTari WG containing *Bacillus thuringiensis* subsp. *aizawai s*train ABTS 1857 will be applied to indoor and outdoor vegetables to control lepidopterous insect pests.

The GAP for indoor and outdoor vegetables is a maximum of 8 applications of 0.540 kg MPCA/ha (corresponding to 1.7 x 10^{13} CFU/ha), with a typical application interval of 7 days (minimum interval 6 day).

Assumptions:

- incorporation into the top 5 cm layer (= 50 L soil/m^2)
- soil density of 1.5 g/cm³ (= 75 kg soil/ m^2)
- no degradation (worst-case)

Indoor and outdoor vegetables

Rate:	26.5 g cry protein/ha (based on a crystalline protein content of 4.9%)
No. applications:	8 (maximum)
Interception:	0%
Spray interval:	6 days minimum

The concentration of the crystalline protein in soil after the final (8^{th}) application in year one was taken as the initial PECsoil, 0.277 mg cry protein/kg dw (dry weight) soil, assuming no degradation; this value has been used in the risk assessment. The PECsoil immediately after the first application was calculated as follows.

$$\text{Initial PECsoil (mg/kg)} = \frac{\text{A (g/ha)}}{100 \times \text{d (cm)} \times \rho\ (\text{g/cm}^3)}$$

where: A = effective application rate (after adjusting for any crop interception)
d = depth of soil layer (5 cm)
ρ = soil bulk density (1.5 g/cm^3)

The potential for soil accumulation has also been considered. This has been addressed by calculating the PECsoil after 10 years of applications (also with no degradation as a worst-case scenario). The accumulated concentration of crystalline protein in soil after eight applications per year for 10 years is 2.822 mg/kg dw soil (3.30 x 10^7 CFU/kg). The PECsoil at time t after application is calculated as follows.

$$\text{PECsoil} = \text{Initial PECsoil} \times e^{-kt}$$

where: k = first-order degradation rate constant (days^{-1}) = ln2/DT_{50}

However, it is unlikely that the conditions required for persistence (*i.e.* low biomass, no sunlight) will occur where applications are made. Therefore, it's unlikely that the MPCA and crystalline protein would persist for long, certainly not 10 years.

The above calculations are supplemented to include PECsoil of MPCA.

Based on 8 applications of 540 g MPCA/ha with 0% interception and no degradation in-between applications, the resulting initial concentration in soil would be 5.76 mg MPCA/kg soil (equivalent to 5.3 x 10^8 CFU/kg soil, based on the conversion of 5 x 10^{13} CFU/kg MPCA).

For accumulated concentrations over 10 years, with no consideration for degradation the concentration in soil after repeated annual applications would be 57.6 mg MPCA/kg soil (equivalent to 5.3 x 10^9 CFU/kg soil, based on the revised conversion of 5 x 10^{13} CFU/kg MPCA).

Note: although values were presented in the submitted dossier, conversion of crystalline protein PECsoil to CFU is not appropriate.

Comments RMS: The RMS agrees with the way the notifier calculated the PEDsoil for the MPCA. The used criteria of no growth, all applications at once and no interception are also used by the RMS. The application rate of 1.7 x 10^{13} CFU/ha results in a value of **1.81 x 10^{8} CFU/ kg** soil should be used for the ecotoxicological risk assessment. This is lower than the value calculated by the notifier of 5.3 x 10^{8} CFU/ kg soil. The RMS has the opinion that the notifier used 5.0 x 10^{13} CFU/ha instead of the 1.7 x 10^{13} CFU/ha. Possibly the notifier used the value 5.0 x 10^{13} CFU/kg this is the conversion factor for weight of to CFU.

B.8.1.2 Water

The fate and behaviour of the MPCA in the aquatic environment is evaluated under Point B.8.1.2 of the corresponding active substance dossier. As it is possible to extrapolate the behaviour of the MPCA resulting from use of the formulated product XenTari® WG from the information on the MPCA itself, additional information investigating the fate and behaviour of the formulated product in the aquatic environment of the have not been performed.

Predicted environmental densities in surface water (PEDsw)

Potential transport routes for surface water exposure include drainage, run-off, atmospheric deposition and spray drift. However, given that applications of the XenTari® WG formulation will be made during the spring and summer to the developing and well-established foliage of vegetables, it is considered that drainage and run-off will be minimal and spray deposition and drift will be the most relevant routes of exposure. Worst-case initial predicted environmental densties in surface water (PEDsw) for use in the environmental risk assessment have been calculated based on the proposed uses of the XenTari® WG formulation.

XenTari® WG will be applied to the range of crops according to the GAP.

Bacillus thuringiensis spores have been found occurring naturally in marine sediments (Japan, Maeda *et al*., 2000), dam sediments and waste collection ponds (Jordan, Obeidat, 2008). [RMS comment: Notifier is asked to provide these references and include summaries in the MA section of the risk evaluation with typical natural densities of the spores.] Use of the formulated product XenTari® WG can potentially lead to MPCA reaching surface water during treatments or via soil run-off, therefore the predicted environmental concentrations in surface water (PECsw) are considered.

XenTari® WG is a water dispersible granule formulation (WG) containing the technical material *Bacillus thuringiensis* subsp. *aizawai* strain-ABTS 1857 at 540 g MPCA/kg. XenTari® WG is intended for use as a ground spray for the control of Lepidoptera caterpillars (from when larvae hatch at L1) in indoor and outdoor vegetables. The critical Good Agricultural Practice (GAP) with the representative uses for XenTari® WG is presented in Appendix 1.

A summary of the information on the fate and behaviour in the aquatic environment of the MPCA is provided above.

FOCUS:

The predicted environmental concentrations of XenTari® WG in surface water (PECsw) are calculated using the standardised recommendations of the FOCUS working group on surface water scenarios (FOCUS 2001[1]).

FOCUS Steps 1 and 2:

Predicted environmental concentrations of the MPCA in surface water following treatment of various crops with XenTari WG have been calculated using the procedures recommended by the FOCUS Working group on surface water scenarios (FOCUS 2001) according to Step 1 and Step 2, using the FOCUS Step 1 & 2 calculator (ver 3.2).

Based on the supported GAP and likely agronomic practices for applications to the various crops, the treatment details and agronomic settings used by the FOCUS Steps 1 and 2 calculator are summarised in Table B.8.1.2-01.

Table B.8.1.2-01 Summary of key agronomic input parameters used for FOCUS Step 1 and 2 calculations

Crop	Application rate (g/ha)	Applications no. / interval per season	FOCUS crop scenario	Crop interception	Region and season
Outdoor fruit vegetables: Pepper	540 g MPCA/ha or 26.5 g cry protein/ha[1]	8 / 6 d	Vegetables, fruiting	No interception	NEU, Mar-May SEU, Mar-May
Indoor fruit vegetables: Pepper	540 g MPCA/ha or 26.5 g cry protein/ha[1]	8 / 6 d	Vegetables, fruiting	No interception	NEU, Mar-May SEU, Mar-May

[1] Based on a cry protein content of 4.9% (arithmetic mean) as quantified by Coddens (1990)

[1] FOCUS (2001): "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.

The chemical parameters of the MPCA used in the calculations are presented in Table B.8.1.2-02.

Table B.8.1.2-02 Summary of endpoints and input chemical parameters for Step 1 and Step 2 simulations

Endpoint	Parameter value	Remarks
Physico-chemical parameters		
Chemical name	Cry protein	
Water solubility (mg/L)	1000 (20°C)	Worst-case assumption
Environmental behaviour		
DT_{50} in soil (days)[1]	i) 2.7 (20°C) ii) 7.0 (20°C)	i) based on KMA 7.1.1/05 ii) based on KMA 7.1.1/06
Soil adsorption coefficient, K_{FOC} (mL/g)[1]	i) 50 ii) 100 iii) 200 RMS comment: Literature values for Koc can be found for Cry proteins. The notifier is asked to include these reference in the MA part and use these values for the protiens and recalculate the PECsw for the Cry proteins with an interval of 6 days	Range of K_{FOC} values tested to demonstrate safe use
Half-life water (days, 20ºC)	1000	Worst-case assumption
Half-life sediment (days, 20ºC)	1000	Worst-case assumption
Half-life sediment/water system (days, 20ºC)	1000	Worst-case assumption

[1] A bracketed approach, using a range of DT_{50} and K_{OC} values has been used to investigate the potential effect of uncertainty in the values.

Using the agronomic information supplied in Table B.8.1.2.-01 and the endpoints and chemical parameters specified in Table B.8.1.2-02, the predicted environmental concentrations of the MPCA crystalline protein following use of the formulated product XenTari WG to treat the worst-case crop according to FOCUS Step 1 and 2 calculations are presented in Table B.8.1.2-03.

Table B.8.1.2-03 FOCUS Step 1 and Step 2 PECsw (µg/L) and PECsed (µg/kg) following use of XenTari WG

Crop (FOCUS crop scenario)	Step	Application rate	EU Region and season of application	Predicted environmental concentrations			
				Multiple		Single	
				PEC_{SW} (µg/L)	PEC_{SED} (mg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (mg/kg)
i) DT_{50} 2.7 days, K_{OC} 50 mL/g							
Vegetables, fruiting 8 x 540 g MPCA/ha (6 day minimum interval)	1	540 g MPCA/ha equivalent to a loading of 26.5 g cry protein/ha	NEU, Mar-May	68.20	34.02	-	-
			SEU, Mar-May	68.20	34.02	-	-
	2		NEU, Mar-May	1.71	0.85	0.83	0.41
			SEU, Mar-May	2.43	1.20	1.42	0.71
ii) DT_{50} 2.7 days, K_{OC} 100 mL/g							
Vegetables, fruiting 8 x 540 g MPCA/ha (6 day minimum interval)	1	540 g MPCA/ha equivalent to a loading of 26.5 g cry protein/ha	NEU, Mar-May	64.30	64.03	-	-
			SEU, Mar-May	64.30	64.03	-	-
	2		NEU, Mar-May	1.63	1.59	0.78	0.77
			SEU, Mar-May	2.30	2.26	1.34	1.33
iii) DT_{50} 2.7 days, K_{OC} 200 mL/g							
Vegetables, fruiting 8 x 540 g MPCA/ha (6 day minimum interval)	1	540 g MPCA/ha equivalent to a loading of 26.5 g cry protein/ha	NEU, Mar-May	57.74	114.58	-	-
			SEU, Mar-May	57.74	114.58	-	-
	2		NEU, Mar-May	1.49	2.85	0.71	1.38
			SEU, Mar-May	2.09	4.05	1.21	2.38
iv) DT_{50} 7.0 days, K_{OC} 50 mL/g							
Vegetables, fruiting 8 x 540 g MPCA/ha (6 day minimum interval)	1	540 g MPCA/ha equivalent to a loading of 26.5 g cry protein/ha	NEU, Mar-May	68.20	34.02	-	-
			SEU, Mar-May	68.20	34.02	-	-
	2		NEU, Mar-May	3.22	1.60	1.35	0.67
			SEU, Mar-May	5.44	2.71	2.46	1.23
v) DT_{50} 7.0 days, K_{OC} 100 mL/g							
Vegetables, fruiting 8 x 540 g MPCA/ha (6 day minimum interval)	1	540 g MPCA/ha equivalent to a loading of 26.5 g cry protein/ha	NEU, Mar-May	64.30	64.03	-	-
			SEU, Mar-May	64.30	64.03	-	-
	2		NEU, Mar-May	3.05	3.01	1.27	1.26
			SEU, Mar-May	5.14	5.10	2.32	2.31
vi) DT_{50} 7.0 days, K_{OC} 200 mL/g							
Vegetables, fruiting 8 x 540 g MPCA/ha (6 day minimum interval)	1	540 g MPCA/ha equivalent to a loading of 26.5 g cry protein/ha	NEU, Mar-May	57.74	114.58	-	-
			SEU, Mar-May	57.74	114.58	-	-
	2		NEU, Mar-May	2.76	5.39	1.14	2.26
			SEU, Mar-May	4.63	9.13	2.08	4.14

Following use of XenTari® WG according to the supported GAP, the maximum potential concentration of the cry protein in surface water and sediment, according to FOCUS Step 2 calculations is 5.44 µg/L and 4.14 mg/kg, respectively (note: the critical values result from multiple applications).

A summary of the results are presented in Table B.8.1.2-04.

Table B.8.1.2-04 FOCUS Step 1 and Step 2 PECsw (µg/L) and PECsed (µg/kg) following use of XenTari® WG

Scenario	Maximum PEC_{SW} (µg/L)		Maximum PEC_{SED} (mg/kg)	
	Step 1	Step 2	Step 1	Step 2
i) DT_{50} 2.7 days, K_{OC} 50 mL/g	68.20	2.43	34.02	1.20
ii) DT_{50} 2.7 days, K_{OC} 100 mL/g	64.30	2.30	64.03	2.26
iii) DT_{50} 2.7 days, K_{OC} 200 mL/g	57.74	2.09	114.58	4.05
iv) DT_{50} 7 days, K_{OC} 50 mL/g	68.20	5.44	34.02	2.71
v) DT_{50} 7 days, K_{OC} 100 mL/g	64.30	5.14	64.03	5.10
vi) DT_{50} 7 days, K_{OC} 200 mL/g	57.74	4.63	114.58	9.13

Additional calculations are provided below to show the concentration of MPCA in adjacent surface waters due to spray drift alone.

Surface water input *via* spray drift was calculated using the following method:

The loading of surface waters due to spray drift is calculated using the spray drift tables of Rautmann *et al.* (2001)[2]. The initial predicted environmental concentrations of the active substance in surface water (PEC_{SW}) are calculated using the following formulae:

PEC_{sw} (initial, single application) (µg/L):	$PEC_{sw\ (ini)} = \frac{A \times dep\ rate}{V_{sw}}$

Where:

$PEC_{SW\ (ini)}$ = PEC in surface water (µg/L) immediately following a single application or following multiple application with no degradation taken into account

A = Application rate (µg/m^2)

dep rate = % spray drift (values used given in tables, based on Rautmann et al, 2001) i.e. 0.277 used to represent 2.77%. A drift value of 0.1% is used for glasshouse applications.

V_{SW} = Water volume of water body per 1 m length of field, i.e. of width 1 m and depth 0.3 m (300 L)

(Note – the calculation equation provided in the admissibility check reporting table could not be followed. The above equation is assumed to be equivalent).

[2] Rautmann, D., Streloke, M. and Winkler, R. (2001): New basic drift values in the authorisation procedure for plant protection products. In: Workshop on Risk Assessment and Risk Mitigation Measures in the context of the Authorisation of Plant Protection Products (WORMM; R. Forster., Streloke, M. Eds), 27-29 September, 1999, Heft 383, BBA, Berlin and Braunschweig, Germany.

It is noted that this general approach more accurately follows the methodology proposed by OECD guidance to the Environmental safety evaluation of microbial biocontrol agents, (Series on Pesticides, no. 67), Feb 2012.

Table B.8.1.2-05 PECsw for outdoor uses via spray drift

Spray drift scenario – arable crops										
Buffer distance	Single application					Multiple applications (n=8)				
	No buffer (1 m)	5 m	10 m	15 m	20 m	No buffer (1 m)	5 m	10 m	15 m	20 m
Drift (%)	2.77[1]	0.57[1]	0.29[1]	0.22[1]	0.15[1]	1.52[2]	0.31[2]	0.16[2]	0.11[2]	0.08[2]
Initial PECsw (µg MPCA/L)										
Field use (peppers), 8x 540 g MPCA/ha	4.99 (or 4.62x 10^5 CFU /L)	1.03 (or 9.50x 10^4 CFU /L)	0.52 (or 4.83x 10^4 CFU /L)	0.36 (or 3.33x 10^4 CFU /L)	0.27 (or 2.50x 10^4 CFU /L)	21.89 (or 2.03x 10^6 CFU /L)	4.46 (or 4.13x 10^5 CFU /L)	2.30 (or 2.13x 10^5 CFU /L)	1.58 (or 1.47x 10^5 CFU /L)	1.15 (or 1.07x 10^5 CFU /L)
Initial PECsw (µg Cry protein/L)[3]										
Field use (peppers), 8x 26.5 g Cry protein/ha	0.244	0.050	0.026	0.018	0.013	1.073	0.402	0.205	0.141	0.106

(1) for a single application 90^{th} percentile drift values are used
(2) for multiple applications (n=8) 67^{th} percentile drift values are used
(3) PECsw for the crystalline protein is calculated on the basis of 4.9% crystalline protein content of MPCA.
Note: conversion of crystalline protein PECsw to CFU is not appropriate.
Conversion to CFU/L is based on 1 µg MPCA equivalent to 9.26x 10^4 CFU.

Table B.8.1.2-06 PECsw for indoor uses via spray drift

Spray drift scenario – glasshouse crops										
Buffer distance	Single application					Multiple applications				
	No buffer (1 m)	5 m	10 m	15 m	20 m	No buffer (1 m)	5 m	10 m	15 m	20 m
Drift (%)	0.1	-	-	-	-	0.1	-	-	-	-
Initial PECsw (µg MPCA/L)										
Glasshouse use (peppers), 8x 540 g MPCA/ha	0.18 (or 1.67x 10^4 CFU /L)	-	-	-	-	1.44 (or 1.33x 10^5 CFU /L)	-	-	-	-
Initial PECsw (µg Cry protein/L)[1]										
Glasshouse use (peppers), 8x 26.5 g Cry protein /ha	0.009	-	-	-	-	0.071	-	-	-	-

(1) PECsw for the crystalline protein is calculated on the basis of 4.9% crystalline protein content of MPCA.
Note: conversion of crystalline protein PECsw to CFU is not appropriate.
Conversion to CFU/L is based on 1 µg MPCA equivalent to 9.26x 10^4 CFU.

As requested, the PECsw of MPCA are also converted to CFU/L for comparative purposes.

Comments RMS:

For products based on micro-organisms the RMS has the opinion that PEDsw should solely be based on the CFU/ha applied to the field. As an extreme worst case all applications are applied at once. Rautmann drift values are applied and a TOXSWA standard ditch of 210 l/m^2 is used. The notifier used FOCUS step 1 and 2 approach for a product with a microorganism as active and also applied it for the endotoxins that are present in the product. The FOCUS scenarios are based on chemical substance properties that do not apply for micro-organisms but can be used for the endotoxins/proteins.

The RMS recalculated PEDsw for eight applications in fruiting vegetables of 1.7 x 10^{13} CFU/ha. As drift values of 1.52% based on Rautmann drift values for seven (or more) applications on fruit crops and a TOXSWA standard ditch of 210 L/m^2 results in a PEDsw of 9.84 x 10^5 CFU/L. This value can be used for the ecotoxicological risk assessment.

B.8.1.3 Air

The fate and behaviour of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS 1857 in the atmosphere was evaluated during the Annex I Inclusion. No additional studies have been performed and no further data are provided. The fate and behaviour of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS 1857 in air is discussed in detail in the corresponding document of the EU review dossier where the references cited from the scientific literature can be found. A brief overview of this information is provided below.

Following application of the XenTari® WG formulation as proposed, spray drift can occur which may result in temporary concentrations of the microbial pest control agent in the atmosphere. However, *Bacillus thuringiensis* subsp. *aizawai* is expected to undergo rapid degradation in the atmosphere since inactivation by solar radiation is a very important factor causing loss of activity and degradation of bacteria spores and δ-endotoxin crystals in the field environment. The survival and persistence of *Bacillus thuringiensis* subsp. *aizawai* in air is therefore expected to be very limited.

Comments RMS: The RMS agrees with the notifier that exposure of air is limited and survival of the micro-organisms in air is not expected.

B.8.2 Mobility

Predicted environmental concentrations in groundwater (PECgw)

The following groundwater scenarios have been simulated to comply with the data gap identified by EFSA with regards *Bacillus thuringiensis* subsp. *aizawai* crystalline proteins (δ-endotoxins), or any of their transformation products retaining insecticidal activity, possibly reaching groundwater.

It is very hard to imagine that the use *Bacillus thuringiensis* as a microbial pest control agent will be able to contaminate ground water at an extent exceeding the "contamination" affected by its natural occurrence. *Bacillus thuringiensis* or relatives have not been considered problematic for drinking water. With regard to groundwater contamination it has to be noted that the crystalline proteins (δ-endotoxins) do not present any risk for human health. Evidence for this is provided by all toxicity studies submitted for the strains. As concluded by EFSA, all *Bacillus thuringiensis* subsp. *aizawai* products contain viable spores and crystalline proteins (δ-endotoxins) associated to the spores. Thus, in all studies, assessing the effect of technical material or formulated products, the test animals have been exposed to the spores and the crystalline proteins (δ-endotoxins). It is therefore not correct that it is concluded by the EFSA that no data are available for the crystalline proteins (δ-endotoxins). As almost all studies did not show adverse effects in the test animals due to exposure to the spores and the associated crystalline proteins (δ-endotoxins) it is not correct to conclude that the crystalline proteins (δ-endotoxins) are of toxicological concern.

Not only due to the nature of *Bacillus thuringiensis* subsp. *aizawai* crystalline proteins (δ-endotoxins) and their environmental behaviour but also for practical issues the FOCUS groundwater model is not strictly applicable for crystalline proteins (δ-endotoxins). The model is designed for assessment of organic compounds. Parameters like vapour pressure, or the K_{OC} value as well as half-life times and water solubility need to be known for using the model and most of them are unknown or not measurable for crystalline proteins (δ-endotoxins). Adoption of default values does not lead to reasonable results. It appears reasonable that this point should be considered as "addressed" by available literature data.

Bacillus thuringiensis is not expected to be mobile in soil and is unlikely to leach through soils to groundwater. The potential for groundwater contamination following application of the XenTari® WG formulation as proposed is therefore negligible.

Various experiments examining the movement of *Bacillus thuringiensis* in soils following spraying of commercial products containing *Bacillus thuringiensis* showed little or no movement. Even one year following an application onto a sandy clay loam soil in a cabbage field in Denmark, 77% of recovered *Bacillus thuringiensis* remained in the 0 to 2 cm topsoil layer (Pedersen *et al.*, 1995). In experiments in Japan, Akiba (1991) found that under artificially and naturally irrigated conditions, there was no translocation of sprayed *Bacillus thuringiensis* into the soil down to a depth of 10 cm. Artificial irrigation with 450 mm simulated rainfall in a soil column showed no movement through 6 cm of volcanic ash and only a few bacteria were detected in the flow through water from movement through a 6 cm column of alluvium sand.

Use of the formulated product XenTari® WG can potentially lead to MPCA reaching groundwater via soil; as such, predicted environmental concentrations in groundwater (PECgw) have been calculated in order to demonstrate safe use.

XenTari® WG is a water dispersible granule formulation (WG) containing the technical material *Bacillus thuringiensis* subsp. *aizawai* Strain ABTS 1857 at 540 g MPCA/kg). XenTari® WG is intended for use as a ground spray for the control of Lepidoptera caterpillars (from when the larvae hatch at L1) in indoor and outdoor fruit vegetables. The critical Good Agricultural practice (GAP) for XenTari® WG is presented in Appendix 1.

The predicted environmental concentrations of relevant components in groundwater (PECgw) following use of XenTari® WG are calculated in accordance with the recommendations of the FOCUS groundwater working group (FOCUS 2000[3] and 2009[4]) using the PEARL (FOCUS version 4.4.4, April 2006) model.

Methodology

Based on the supported GAP and likely agronomic practices, applications to the various crops were simulated as summarised in Tables B.8.2.1-01 and B.8.2.1-02 where the rationale for all selected parameters is clearly explained.

[3] FOCUS (2000) "FOCUS groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp

[4] FOCUS (2009) "Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 1, 604 pp.

Table B.8.2-01 Application dates modeled in the leaching simulation model PEARL

Crop	**Tomatoes**
Application rate (g as/ha)	26.5[1]
Number of applications/interval	8 applications
Application interval	7 days
Relative application dates	*Application 1:* 14 days post-em *Application 2:* 21 days post-em *Application 3:* 28 days post-em *Application 4:* 35 days post-em *Application 5:* 42 days post-em *Application 6:* 49 days post-em *Application 7:* 56 days post-em *Application 8:* 63 days post-em
Crop interception (%)	0
Frequency of application	Annual
Models used for calculation	FOCUS PEARL v4.4.4

[1] Based on a cry protein content of 4.9% (arithmetic mean) as quantified by Coddens (1990)

Table B.8.2-02 Application dates modeled in the leaching simulation model PEARL

Scenario / Crop	**Application[1] dates modelled**								
	Default date	**Appln 1**	**Appln 2**	**Appln 3**	**Appln 4**	**Appln 5**	**Appln 6**	**Appln 7**	**Appln 8**
Tomatoes									
Châteaudun	10-May	24-May	31-May	07-Jun	14-Jun	21-Jun	28-Jun	05-Jul	12-Jul
Piacenza	10-May	24-May	31-May	07-Jun	14-Jun	21-Jun	28-Jun	05-Jul	12-Jul
Porto	15-Mar	29-Mar	05-Apr	12-Apr	19-Apr	26-Apr	03-May	10-May	17-May
Sevilla	15-Apr	29-Apr	06-May	13-May	20-May	27-May	03-Jun	10-Jun	17-Jun
Thiva	10-Apr	24-Apr	01-May	08-May	15-May	22-May	29-May	05-Jun	12-Jun
Notes: The default dates refer to the FOCUS date for transplanting at each scenario location. Based on the information provided for the application timings, the first application for all the relevant scenarios was taken to be 14 days after the default date (*i.e.* the earliest treatment dates possible was assumed to represent a worst-case); following treatments were made using the minimum interval of 7 days. Treatments were assumed to be conducted each year. The crop type cabbage within the models allows for two crop cycles per season. Within the model, relative applications dates were used *i.e.* emergence + 14, 21, 28, 35, 42, 49, 56, 63 days.									

The endpoints and model inputs relating to the chemical parameters used for the determination of the predicted environmental concentrations of the cry protein in groundwater are summarised in Table B.8.2-03.

Table B.8.2-03 Summary of endpoints and chemical input parameters for the leaching simulation model PEARL

Endpoint	**Parameter value**	**Remarks**
Physico-chemical parameters		
Chemical name	Cry protein	
Molecular weight (g/mol)	1000	Worst-case assumption
Vapour pressure (Pa)	1.0×10^{-9} (20°C)	
Molar enthalpy of vaporization (kJ/mol)	95	FOCUS recommendation
Water solubility (mg/L)	1000	Worst-case assumption
Molar enthalpy of dissolution (kJ/mol)	27	FOCUS recommendation
Diffusion coefficient in water (m²/d) (m²/s)	4.3×10^{-5} (20 °C) (PEARL) 5.0×10^{-10} (20°C) (MACRO)	FOCUS recommendation
Diffusion coefficient in air (m²/d)	0.43 (20°C)	FOCUS recommendation
Degradation in soil		
DT_{50} in soil (days)	i) 2.7 ii) 7.0	i) based on KMA 7.1.1/05 (MM-A) ii) based on KMA 7.1.1/06 (MM-A)
Temperature correction function: - reference temperature (°C) - molar activation energy (kJ/mol)	20 65.4 (PEARL)	FOCUS recommendation
Sorption to soil		
Soil adsorption coefficient, K_{FOC} (mL/g)	i) 50 ii) 100 iii) 200 (pH independent PEARL)	Range of K_{FOC} values tested to demonstrate safe use
K_{FOM} (mL/g)[1]	i) 29 ii) 58 iii) 116	Range of K_{FOM} values tested to demonstrate safe use
Molar enthalpy of sorption (kJ/mol)	0	FOCUS recommendation
Freundlich exponent 1/n (-)	0.9	FOCUS default
Reference concentration in liquid phase (mg/L)	1	FOCUS recommendation
Desorption rate coefficient (d^{-1})	0	FOCUS recommendation
Factor relating CofFreNeq and Cof-FreEql (-)	0	FOCUS recommendation
Crop/management related parameters		
Plant uptake factor	0	Not specified in LoE. FOCUS default for non-systemic substance
Application depth (cm)	0	FOCUS recommendation
Other model specific parameters		
Wash off factor (m^{-1})	0	FOCUS recommendation
Canopy process option	Lumped	FOCUS recommendation
Half-life at crop surface (d)	1000000	FOCUS recommendation

[1] Derived from K_{FOC} value divided by 1.724

Using the agronomic parameters specified in Tables B.8.2-01 and B.8.2-02 and the endpoints and chemical parameters specified in Table B.8.2-03 for the component of interest cry protein, simulations were conducted to determine the predicted 80th percentile concentration of the MPCA. The results are summarised in Table B.8.2-04.

Table B.8.2-04 Predicted groundwater concentrations of cry protein following application of XenTari WG to treat fruit vegetables using FOCUS PEARL 4.4.4

Scenario	**Maximum 80th percentile annual average concentration (µg/L)**					
	i) DT$_{50}$ 2.7 d K$_{OC}$ 50 mL/g	**ii) DT$_{50}$ 2.7 d K$_{OC}$ 100 mL/g**	**iii) DT$_{50}$ 2.7 d K$_{OC}$ 200 mL/g**	**iv) DT$_{50}$ 7 d K$_{OC}$ 50 mL/g**	**v) DT$_{50}$ 7 d K$_{OC}$ 100 mL/g**	**vi) DT$_{50}$ 7 d K$_{OC}$ 200 mL/g**
Outdoor and Indoor Fruit Vegetables (Tomatoes)						
Châteaudun	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Piacenza	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Porto	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sevilla	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Thiva	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Using the FOCUS methodology, the 80th percentile PECgw values of the component of interest cry protein in groundwater were generated assuming repeated annual applications at the maximum seasonal use rate for the worst-case crops identified. Annual average concentrations were calculated as the cumulative annual chemical flux divided by the cumulative annual water recharge volume at 1 m depth. The predicted concentration is a conservative estimate of what may actually be expected in groundwater used for drinking water as soil pore water at one-meter depth is not a likely source of drinking water.

In the reasonable worst-case FOCUS scenarios modelled, the annual average concentration of the component of interest cry protein in soil pore water at one-meter depth following use of XenTari WG was less than 0.1 µg/L.

The results from this modelling study indicated that the leaching potential of the component of interest cry protein is low (80th percentile PECgw <0.1 µg/L) under all FOCUS leaching scenarios using the maximum application rates applied to any crops.

Comments RMS: The RMS understands the method used by the notifier to calculate the PECgw for the cry proteins. The composition and amount of the cry proteins may vary in the product. The amount of cry protein in the product is low. Additionally, the notifier missed some essential scientific literature on the adsorption/desorption of the cry proteins. However, for a product based on a micro-organism the approached used is not valid. Leaching of the micro-organism is not expected since the mobility is limited (see CA part of the RAR).

B.8.3 References relied on

No reference submitted for this part of the dossier.